

BIODEGRADATION OF VINYL SULFONE REACTIVE DYES

A THESIS

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BIODEGRADATION OF VINYL SULFONE REACTIVE DYES

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DEDICATION

I dedicate this work to my wife, Pat, and daughter, Amanda, for their constant support and encouragement throughout this endeavor.

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SUMMARY

Biological degradation of three vinyl sulfone class fiber reactive dyes, widely used for dyeing cotton, was investigated under laboratory conditions simulating those employed in conventional activated sludge waste treatment plants. The three dyes studied were Reactive Blue 19, Reactive Violet 5, and Reactive Black 5.

Chromatographic and spectrophotometric analysis of the digestion products recovered from the aerobic systems failed to show any evidence of degradation of the dyes. All three of the dyes remained in solution in the aqueous phase throughout the 20 day retention period in the digester with none being adsorbed on the sludge floc.

Reactive Blue 19 and Reactive Violet 5 were also studied under anaerobic conditions. Both dyes were degraded as evidenced by chromatographic and spectrophotometric analysis of the degradation products recovered from the effluent.

The rapid disappearance of color in the supernatant liquid in the anaerobic digester indicated that the chromophoric group of the Reactive Violet 5 was destroyed. Subsequent analysis revealed two colorless metabolites which were not present in sufficient quantities for identification.

Reactive Blue 19 was partially degraded to yield five colored metabolites, the principal one of which had a violet color. The four other metabolites, two of which were yellow and two orange, all

fluoresced under ultraviolet light. All four were present in insufficient quantities for identification.

The violet metabolite was extracted with ether from the concentrated sludge layer with none being extracted from the supernatant layer. The violet metabolite was soluble in ether and acetone and insoluble in water.

Comparison of the infrared spectrum of the violet metabolite with that of a known disperse dye, Disperse Blue 9 (C.I. 61115), led to the conclusion that the generic chemical structures of the two were very similar.

CHAPTER I

INTRODUCTION

One of the greatest problems facing the world today is the prevention of pollution of its fresh water resources. In the past decade population along specific river basins, accompanied by industrial expansion, has greatly increased. The struggle for usable water continues to plague many municipalities and industries in their daily operations. The quantity of water available does not keep pace with the demand, and accordingly the quality of the water available declines (11).

Fortunately, in Georgia the problem has not yet become critical. William H. Weir (24) in addressing a symposium on industrial wastes in 1955, invited industry into Georgia because the streams of Georgia were so pure that the new companies would not have to worry about polluting them. Weir stated that the method of stream control used in Georgia at that time was to monitor the streams after the industries had located, rather than to impose specific pollution control standards on them before location.

Certainly, the methods have changed in recent years due directly to the increase in urbanization and industrial expansion.

Only two decades ago the primary interest in organic contaminants in surface waters was essentially limited to taste and odor in potable water (19).

This was evident in the 1946 United States Public Health Service

Drinking Water Standards which recommended that "the concentration of phenolic compounds should not exceed 0.001 milligrams per liter (mg/l)," and also that "drinking waters should have no objectionable taste or odor." Reports concerning contamination of surface waters by DDT, foaming caused by the presence of synthetic detergents in lakes and streams, and taste and odor were just beginning to be published (19).

The effects of organic contaminants on surface water uses may be divided into three principal areas--effects on man, streams, and industrial processes.

Among the effects on man, taste and odor of his drinking water are foremost. Objectionable taste and odor may arise from specific chemicals present to the extent of one or more micrograms per liter. Toxaphene, a commonly used pesticide, has a threshold odor value of five micrograms per liter, and parathion, another pesticide, has a threshold odor value of only one microgram per liter (19).

In regard to man's health, there is little or no published data covering physiological effects resulting from organic materials in the concentrations now reported in waters, but the health hazard to man is potentially present (19).

The effects of organic contaminants on the ecology of the streams receiving them range widely and are sometimes more readily observed than they are on man. In addition to a possible change in the color of the water there may be a very large dissolved oxygen loss or an exertion of biochemical oxygen demand, thus making the water an unsuitable environment for many living organisms. Some of the contaminants entering the waters may be lethal to the fish. Lindane, for example, has a lethal

limit of 77 micrograms per liter to bluegill fingerlings (19).

The flesh of fish which do survive in contaminated waters may become unfit for consumption. Animal and waterfowl populations in these areas may be seen to decrease very sharply (19).

Most important of all to the survival of a healthy stream, the microbial purification cycle becomes upset, and eventually the waters become septic in stagnant zones (19).

The effects of organic contaminants on waters for industrial usage range as widely as the industries themselves. They may range from the spoiling of beverages or the limiting of chemical reactions or the changing of dye shades. No matter what the case, the result is reflected in an increase in water treatment costs and eventually an increase in costs to the consumer (19).

Statement of the Problem

The textile industry is the largest industry in Georgia today, employing more than 93,000 workers (3), and at the present time is its third largest user of process water excluding paper. A twofold increase in textile production in some areas over the next two years has been forecasted (1).

The discharge of dyes from dyehouses into the streams, with resultant color buildup, has long been one of the main concerns of the industry.

Coloration of the stream alone does not indicate the composition of the materials carried by the stream, but to the layman a highly colored stream indicates that it is dangerously polluted (8).

Nemerow and Doby (12) reported the study of color removal from dyehouse effluents using photometric methods to determine the efficiency of color removal. At over 50 percent of the plants covered in their study, color persisted in the effluent. Of those plants, less than 20 percent were making any attempt to evaluate the magnitude and significance of the color density.

Hyden, Becknell, and Elders (7) conducted interviews in 48 manufacturing plants in various geographical areas of Georgia in an effort to learn what each industry was doing about the pollution problem. Of the textile mills visited seven discharged raw waste directly into the streams. Four of the others considered their treatment of waste water to be inadequate for their present production levels.

Powell (16) and Pratt (17) have investigated the biological degradation of certain chemical classes of disperse dyes using activated sludge. They found that the dyes were degraded into various aromatic metabolites some of which were highly colored.

Dyes are highly complex organic compounds often closely resembling pharmaceuticals in their structures. This is one of the primary reasons for the growing concern in the ultimate fate of textile mill wastes.

For example, the sulfone and sulfonamide drugs, which are closely related to two of the classes of fiber reactive dyes, can become toxic when not administered properly. Among some of their untoward effects are hemolysis of varying degrees, aplastic anemia, and disturbances of the urinary tract (4).

Tada, Ishidate, and Odashima (23) investigated, for carcinogenic

activity on laboratory mice, several polycyclic quinones, some of which are dye intermediates. The chemicals were administered over several months, and carcinogenic activity was evident in nearly all of the substances studied.

Pliss (15) investigated four azo and diazo dyes and one anthraquinone dye of the vinyl sulfone class of reactive dyes for carcinogenic activity, when administered to mice and rats by mouth or subcutaneously, for various periods up to 26 months. After four to 24 months, malignant tumors of various types and localizations were found in 10 to 65 percent of the surviving animals in the respective groups. Liver sarcoma was the most prevalent type. Only one of the azo dyes was noncarcinogenic. However, it did cause acute hepatic dystrophy. The probable reason for its noncarcinogenic nature was attributed to the fact that it was a stable copper complex and was thus unable to release its free aminonaphthol. All of the dyes studied produced chronic lipid nephrosis in the experimental animals (15).

Delfina and Pozo (2), through successive separations using paper chromatography, succeeded in isolating 11 different coal tar derivatives in commercial Red R (C.I. 16150), a dye used in pharmaceuticals, foods, and cosmetics. Experiments on rats indicated that one of the derivatives, the 2,4-xylylidine derivative, was hepatotoxic and possibly carcinogenic.

Malaney, et al. (10) studied the response of 27 known carcinogenic substances in activated sludge obtained from three locations. Many of the substances used were dye intermediates. In nearly all of the tests the substances resisted oxidation by the sludge during normal retention periods.

Historical Background of Reactive Dyes

Fiber reactive dyes react chemically with cellulosic fibers forming a covalent bond. The reaction product is quite stable and resistant to hydrolysis in a neutral or alkaline medium. Many of the reactive dyes in use today were derived from acid dyes which had been modified to contain a reactive group.

The reactive dyes were easily adapted to the dyeing of cellulosic fibers in blends containing polyester and acrylic fibers. They can be applied by a variety of process techniques, and they are particularly well suited for the dyeing of cotton-polyester blends using a continuous method (18).

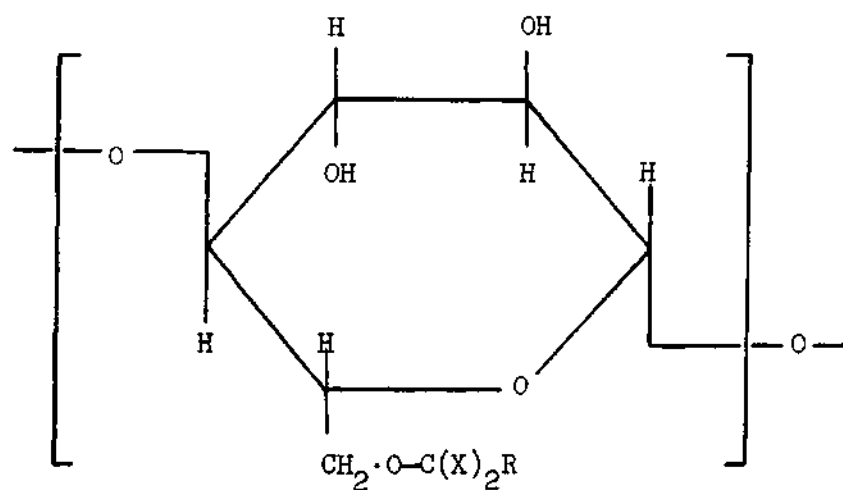
This adaptability coupled with the excellent fastness properties and bright shades obtainable has been the reason for their widespread and increasing usage.

✓ Basically, there are two methods by which a reactive dye molecule may be attached to cellulose, esterification and etherification (Figure 1) (18).

The earliest studies on the esterification of cellulose were made by Cross and Bevan in the 1890's (18). They treated cellulose with strong caustic soda to produce "alkali cellulose." This was further reacted to finally yield a colored derivative (Figure 2).

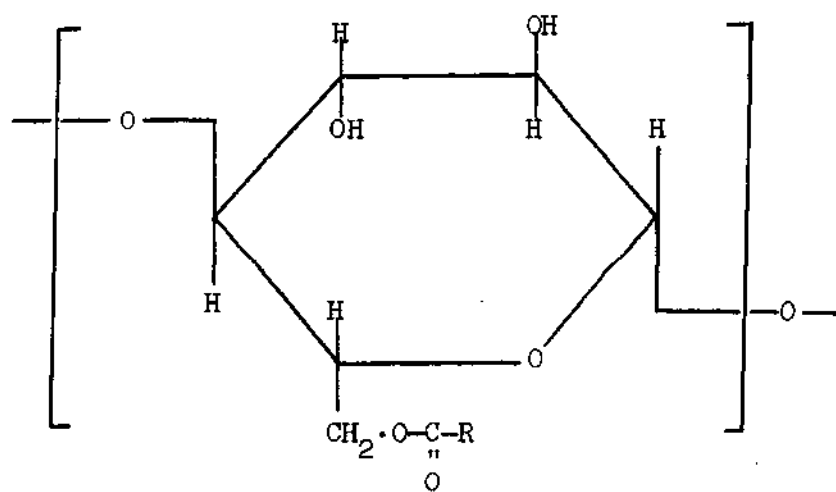
This basic approach to esterify cellulose was used over the next 40 years using different esterifying agents and reaction environments (18). However, all of the methods evaluated were highly unsuitable for practical dyeing processes.

In 1953, Imperial Chemical Industries Limited (I.C.I.) developed



Etherification

X = Hydrogen, Alkyl Group, etc.



Esterification

Figure 1. Methods for Attaching a Dye Molecule to Cellulose (18)

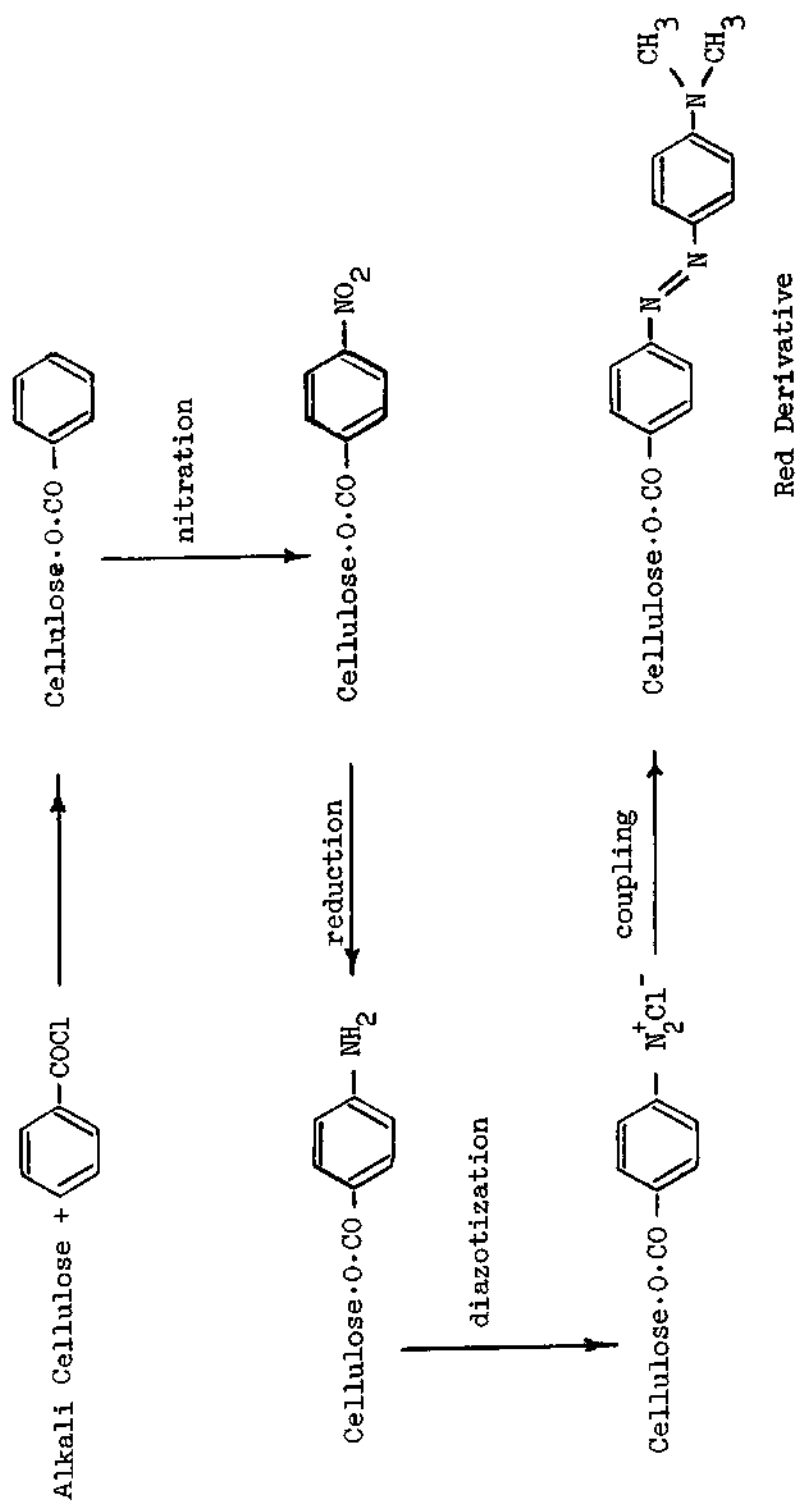


Figure 2. Cross and Bevan Reaction Mechanism for Dyeing Cellulose (18)

a class of fiber reactive dyes designated as "Procions" (18). These were dichlorotriazinyl amino dyes and were reacted in an aqueous solvent with alkali cellulose. Further study showed that the very strong alkaline solutions which had been a feature of all the earlier work were unnecessary, and that a relatively dilute caustic soda solution (pH 7.5 to 8.5) could be used, especially if the solution was saturated with common salt. This important discovery was to pave the way for reactive dyes to be used in dyeing cellulose in blends with synthetic fibers. These dyes were not formally introduced to the trade until April 1956 (18).

Although the study of the etherification of cellulose with the dye was started much later than the esterification route, it has been more widely investigated.

Peacock (14) achieved moderate success by boiling cotton in an aqueous solution of m-nitrobenzyl, dimethyl, phenylammonium chloride to form the m-nitrobenzyl ether. This he reduced, diazotized, and coupled with various naphthol sulphonic acids to give fast to washing pink to fawn shades.

Guthrie (5) was the first to use dyes directly as etherifying agents. He impregnated cotton with "Solacet Fast Yellow GS" dissolved in a strong caustic soda solution (10 to 30 percent). By drying the impregnated cotton for one hour at 100 to 120 degrees Centigrade ($^{\circ}\text{C}$), he found that fast dyeings could be achieved. He also found that this method gave low dye absorption efficiency, that is, much of the dye in the solution was not taken up by the cotton. Also, upon rinsing the treated and dried fabric he found that a considerable amount of dye was

removed. These two features are characteristic of all fiber reactive dyes. The amount of dye fixed in the cellulose may range from as low as 20 percent to as high as 95 percent.

In 1957, Hoechst A. G. introduced a vinyl sulfone class of reactive dyes known as "Remazols" (18).

XX Actually the term fiber reactive dye does not describe the chemical nature of the dye, but rather refers to the fact that the dye is chemically bonded to the fiber. Fiber reactive dyes may belong to one of many different chemical classes as seen from Figure 3 (6). It can also be seen that the chromaphoric group can range widely enough to encompass many different atomic configurations.

In 1966, only 13 years after fiber reactive dyes were first introduced, the United States' annual production reached 1.9 million pounds. The quantity imported annually is lost in the records as only "Synthetic Dyes," but it may be as large as half of the total United States' production (22).

As stated earlier, low dye efficiency is a characteristic feature of the fiber reactive dyes. Much of the dye entering the dyeing process fails to combine with the fiber and is discharged as process effluent. The unreacted hydrolyzed dye is highly soluble and remains in solution in the effluent.

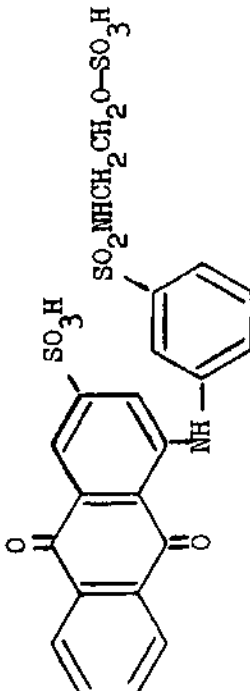
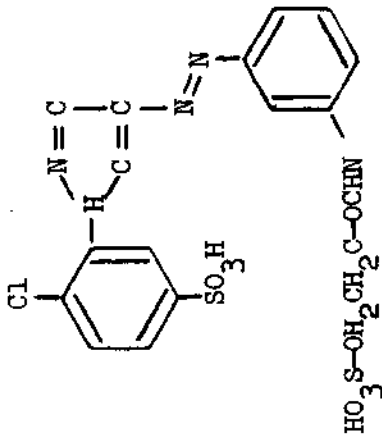
<u>Chemical Name</u>	<u>Reactive Group</u>	<u>Typical Reactive Dye</u>
Vinyl Sulfonamide	$\text{SO}_2\text{NH} - \text{CH}=\text{CH}_2^*$	
Acrylamide	$-\text{NH} - \text{CO} - \text{CH}=\text{CH}_2^*$	

Figure 3. Concluded

CHAPTER II

EXPERIMENTAL PROCEDURE

Selection of Dyes

Three vinyl sulfone fiber reactive dyes were selected for this study. They were Reactive Blue 19, Reactive Violet 5, and Reactive Black 5. These three dyes are widely used in the textile industry. The primary reason for their selection for this study was that their chemical structures were known (Figure 4).

Reactive Blue 19 and Reactive Violet 5 were studied under both aerobic and anaerobic conditions, while the Reactive Black 5 was studied only under aerobic conditions.

Method of Attack

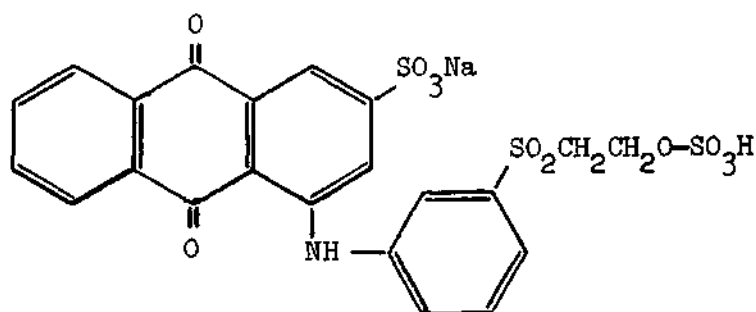
Digestion of the dyes in activated sludge was used as the method of investigation. Both aerobic and anaerobic conditions were covered.

Activated sludge was obtained from the aeration tanks at the Atlanta, Georgia, South River Treatment Plant. Waste input to this plant is primarily of domestic origin.

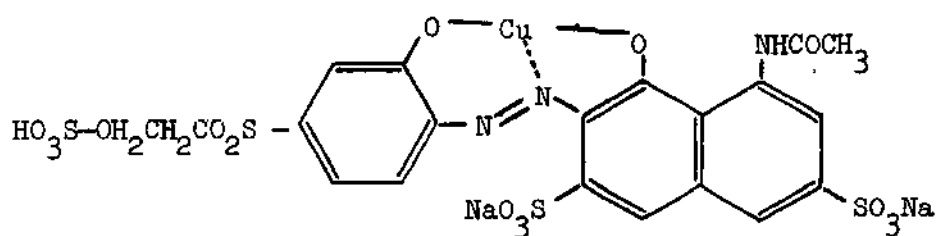
One gallon wide mouth jars were used as aeration tanks (Figure 5).

One gallon small mouth jars were used as anaerobic digesters. The jars were fitted with Neoprene stoppers containing gas exhaust and feed tubes (Figure 6).

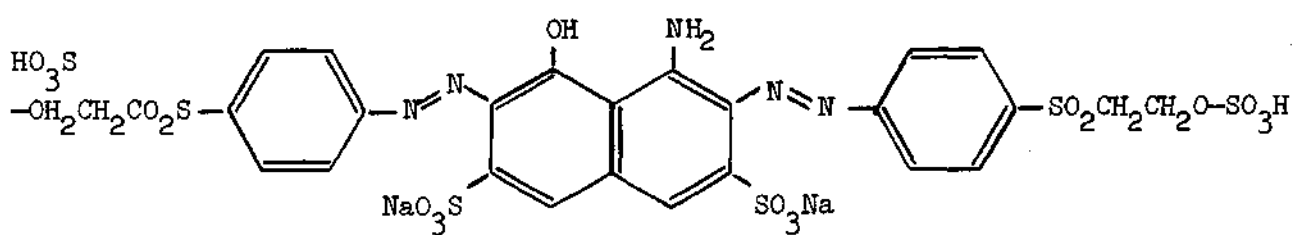
All visible spectra were recorded on the Beckman DB-G Grating Spectrophotometer and 10 inch recorder.



C.I. Reactive Blue 19



C.I. Reactive Violet 5



C.I. Reactive Black 5

Figure 4. Chemical Structures of Dyes Studied (13)

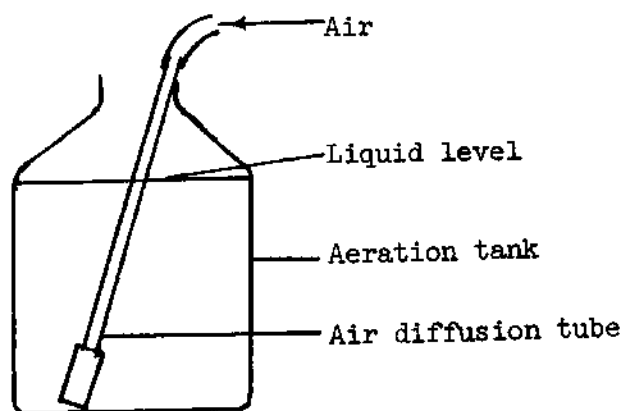


Figure 5. Aerobic Digestion Tank

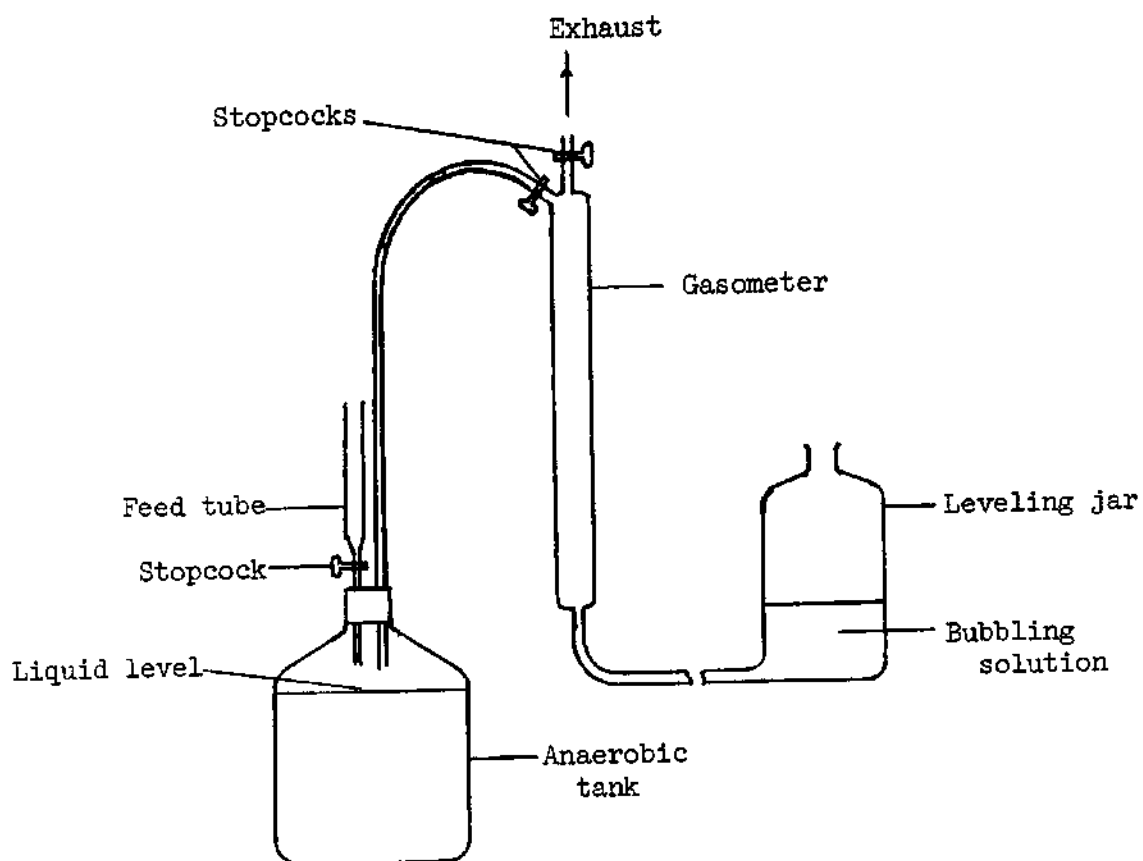


Figure 6. Anaerobic Digestion Tank

Samples were concentrated on the Büchi Rotavapor model R and dried in a National Vacuum Oven at a reduced pressure equivalent to 25 to 29 inches of mercury, i.e. one to five inches absolute pressure.

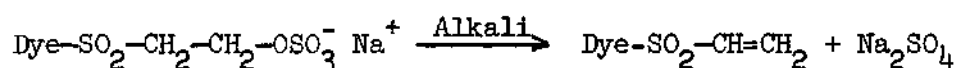
Infrared spectra of the dyes and their metabolites were charted on the Beckman IR 10 Infrared Spectrophotometer from potassium bromide (KBr) pellets.

Five day bacterial counts were made in nutrient agar in disposable Petri dishes. Counts of viable bacteria were made on a Quebec counter after 48 hours incubation at 37°C.

Thin layer chromatography was used to separate the metabolites using Silica Gel G and Desaga tanks with ground glass covers.

Preparation of Dyes

The commercial vinyl sulfone dyes are in a stable sodium salt form in the powder state and only by heating under alkaline conditions is the vinyl sulfone generated (9).



This generated vinyl sulfone can react not only with cellulose, but also with water to give the hydroxyethylene-sulfone as seen below (9).



Thus, dye not reacted with the fiber in the dyeing process reacts with water and remains in solution in the dyebath effluent at the com-

pletion of the dyeing cycle. In a poorly controlled process, unreacted dye may constitute up to 80 percent of the dye fed to the dyebath.

The dyes used were boiled for one hour in a laboratory scale alkaline dyebath. The solution of each dye was cooled to room temperature, and the pH was adjusted to 7.0 to 7.5. The dye solutions were then diluted to sufficient volume to give concentrations of one gram per liter; the solutions were capped and stored in the dark until further use.

Blanks of these prepared dyes were aerated in tap water for 20 days with no change in recorded spectra.

Aerobic Digesters

The activated sludge as obtained from the South River Treatment Plant in Atlanta, Georgia, was filtered through a coarse screen to remove unwanted debris such as leaves and twigs.

The sludge was then acclimated to laboratory conditions in a five gallon reservoir by daily feeding of a mixture of 3:1 dextrose:peptone while aerating. This was continued for at least five days.

After the sludge was acclimated, the desired amount was siphoned from the reservoir during aeration into the one gallon jars. It was allowed to settle for one hour, and the volume of sludge was adjusted to give between 20 and 30 percent of the total liquid volume. The effluent was siphoned off and replaced with tap water.

The hydrolyzed dyes were added to the aeration tanks in three increments over a 48 hour period to give a total concentration of 10 mg/l. As the dyes were added the dextrose and peptone were decreased to zero over three days.

The tanks were aerated for 23 hours with one hour of settling before samples were withdrawn (21). Daily records of pH, temperature, chemical oxygen demand (C.O.D.) (20), and sludge color and volume were kept. In addition, a count of viable bacteria was made at periodic intervals during the 20 day retention period (Tables 1, 2, and 3).

Samples taken for visible spectra readings were first filtered through a Buchner funnel using Whatman #1 filter paper and Celite Analytical Filter Aid. The filtered samples were concentrated on the Rotavapor and then filtered through a micropore filter in order to remove the bacteria. Visible spectra of the solutions were then run on the Beckman DB-G Grating Spectrophotometer at a wavelength ranging from 320 to 750 millimicrons.

At the end of the 20 days, the contents of each tank were extracted with ether. No metabolites could be detected in the ether extract, so the effluent was filtered and condensed as before. A portion of the effluent was evaporated to dryness, and infrared spectra were run on these dried samples recovered from the digestion tanks.

Anaerobic Digesters

Two liters of the acclimated domestic sludge were siphoned into the anaerobic tanks, and the system was closed to exclude air. The sludge was further acclimated to anaerobic conditions by feeding daily 250 milligrams of peptone and 450 milligrams of dextrose per 500 milliliters of sludge.

The system was acclimated for five to seven days in order to deaerate it. Daily records of the volume of gas expelled were read on calibrated gasometers which contained a solution of 150 g/l sodium

chloride and five milliliters per liter (ml/l) of concentrated hydrochloric acid. This solution was used to absorb some of the odor given off by the digesters.

Once the daily gas volumes remained relatively constant, the system was considered acclimated to anaerobic conditions.

Three digesters were used, one as a control and the other two for the dyes studied. All three digesters received daily feedings of the dextrose and peptone mixture with the dye digesters also receiving 10 mg/l daily of the hydrolyzed dyes.

Sufficient supernatant liquid to read the pH of the solution and to dissolve the food was withdrawn from the digesters by tilting the jar and opening the stopcock on the feed tube (Figure 6). The liquid from the control tank was used to dissolve the food for that tank, while in the case of the other two tanks the dye solutions were used for that purpose.

Feeding was accomplished by filling the feed tube with the proper nutrient solution and altering the position of the leveling jar to give a negative pressure inside the tank. The stopcock on the feed tube was slowly opened, and the food solution was sucked into the tank. Extreme care was taken to leave a small amount of the food solution in the tube so that no air reached the system. The leveling jar was returned to its normal elevated position, and the liquid level in the gasometer was marked.

Each tank was then stirred by vigorous shaking immediately following feeding and three additional times during the next eight hours. They were then allowed to stand overnight before the next feeding.

Daily records were kept of room temperature, pH of the tanks, total gas volume given off in 24 hours, and the amount of dye added to the tanks (Table 4).

Although normal retention time for anaerobic systems is 30 days, these tanks were retained for only 10 days due to the rapid disappearance of color in the tanks. The color of the two dye tanks was noted to disappear between three to five hours after feeding. Also, the gas volumes given off seven hours after feeding were 80 to 90 percent of the total volume obtained after 24 hours.

At the end of the retention period the sludge and the supernatant liquid from each tank were neutralized and extracted separately with ether. The ether extract was condensed for use in separating the metabolites.

Isolation of Metabolites

Thin layer chromatography was used to separate the metabolites resulting from the biodegradation. Both five by 20 centimeter (cm) and 20 by 20 cm thin layer plates were prepared with 26 grams of E. R. Merck silica gel G and 52 ml of distilled water. The slurry was swirled vigorously for one minute and applied with a Desaga spreader to a thickness of 250 microns. The plates were air dried at room temperature for one hour, and then they were activated for one hour at 120°C and stored in a desiccator until used.

All plates were spotted 1.5 cm from the bottom of the plate, and the chromatograms were developed to 15 cm with a 50 percent solution of chloroform in acetone in Desaga tanks equipped with ground glass covers.

All metabolites were individually removed from the thin layer plates into 12 by 75 millimeter (mm) test tubes. They were dissolved in acetone, centrifuged to remove the silica gel, and stoppered until further use.

CHAPTER III

DISCUSSION OF RESULTS

Aerobic Study

None of the three dyes subjected to aerobic digestion conditions were degraded by the activated sludge during normal retention time. The daily visible spectra of the dye tank effluents indicated no change in the adsorption curves (Figure 7). Infrared analysis of the dyes recovered from the aerobic digesters gave the same spectrophotometric profile as the dyes as introduced, although there was slight interference from a substance common to the sludge.

All three of the dyes remained in solution throughout the entire period with very little adhering to the floc.

There was a possibility of the dyes being toxic to the bacteria as indicated by a general decrease in the bacterial counts with the exception of the Reactive Black 5.

In that digestion tank, there was a slight increase in the count at the start, which remained steady only to decrease slightly at the end of the retention period. The increase might have resulted from the breakdown of very small bacterial colonies during dilution and mixing. This also could have accounted for the slight increase in the counts from the other two tanks at the end of the retention period.

A second possibility for the increase could have been that a biostatic effect was present and effective in the higher concentrations of

the samples. If this were the case, it would have meant that the dilution of the toxic substances had allowed the number of bacteria in the higher dilution samples to multiply at the same rate as those of the lower dilution samples.

The evaluation of toxicity was inconclusive due to insufficient evidence or the inability of the investigator to properly interpret his data and observations.

Anaerobic Study

Both dyes studied under anaerobic conditions were degraded by the digester sludge.

Reactive Violet 5 was readily reduced to colorless metabolites which were water soluble. Although no metabolites were recovered in sufficient quantities for positive identification, the most likely degradation pathway started with a partial reduction of the copper complexed azo linkage followed by the complete reduction of that linkage and the splitting of the molecule into its two major aromatic components, which may or may not have been further degraded.

Thin layer chromatography of the ether extract from the Reactive Violet 5 tank revealed nothing until the plate was sprayed with Ninhydrin and baked for 15 minutes at 120°C. Two metabolites were revealed at this point in very small quantities. A blue metabolite with an R_f value of 2.3, and a red metabolite with an R_f value of 8.0 were evident. Both metabolites disappeared within 15 minutes of removal of the plate from the oven. Further efforts to extract sufficient quantities for further analysis from the effluent were unsuccessful.

Reactive Blue 19 was degraded into five colored metabolites with various characteristics (Table 5). The violet metabolite was present in sufficient amounts for analysis. It was extracted largely from the concentrated sludge. This was the first indication that it was water insoluble or absorbed on the sludge solids.

The infrared spectrum of the violet metabolite was compared to the infrared spectrum of Disperse Blue (C.I. 61115)(Figure 7).

The spectra had several peaks in common indicating a similarity in structures, which led to a possible degradation pathway for the Reactive Blue 19 (Figure 8).

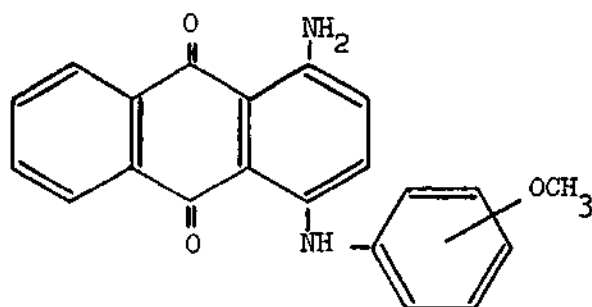


Figure 7. Disperse Blue 9 (C.I. 61115)

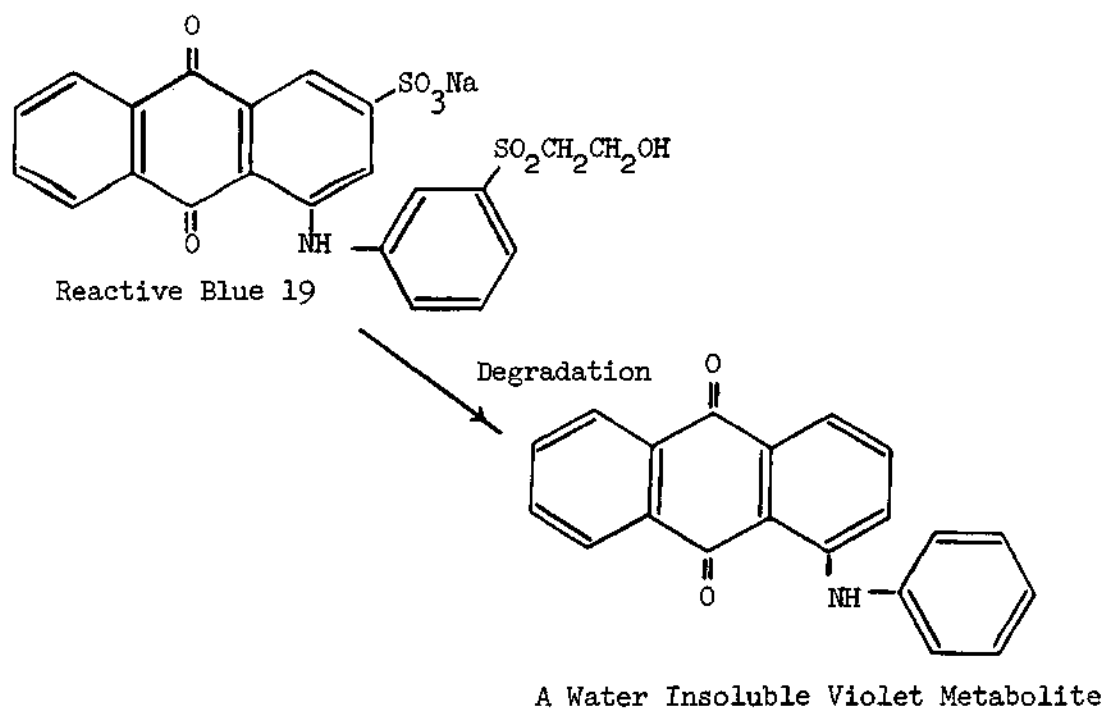


Figure 8. Degradation Pathway for Reactive Blue 19

CHAPTER IV

CONCLUSIONS

The vinyl sulfone reactive dyes investigated were not degraded using conventional aerobic waste treatment methods. They remained in solution in the liquid phase and passed through simulated conventional waste treatment digesters essentially unchanged. The dyes were not toxic, or only moderately so, to the bacteria in the aeration tanks.

The dyes were, however, degraded under anaerobic conditions into various organic metabolites, some of which were still highly colored.

CHAPTER V

RECOMMENDATIONS

Additional degradation studies should be made on all of the classes of the fiber reactive dyes using conventional waste treatment methods. Cooperation of the dye manufacturers should be enlisted in order to have them provide the chemical structures of those dyes that are the most widely used.

Studies should also be made using sludge samples taken from various waste treatment plants in order to determine the activity of sludge used to treat industrial wastes against sludge used primarily to treat domestic wastes.

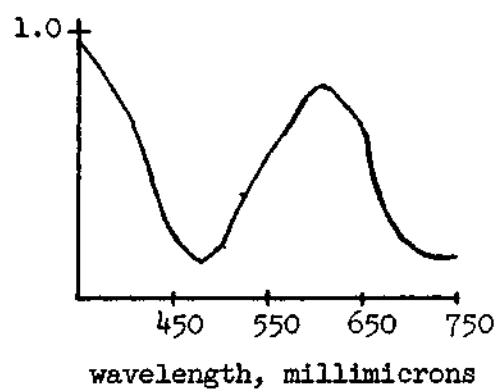
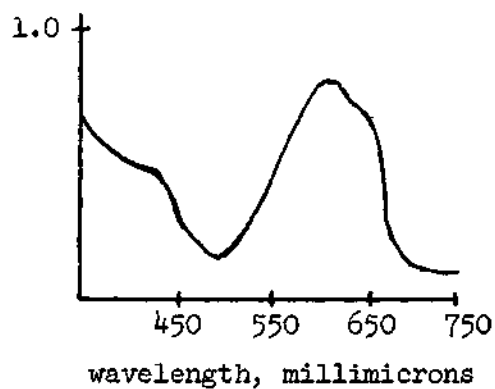
Since some of these dyes are non-responsive to aerobic treatment, a study should be made of the ecology of the streams receiving waste carrying vinyl sulfone dyes. Field studies made on small streams known to carry large volumes of textile wet processing wastes, exclusive of other industrial wastes, should provide much valuable data on stream ecology as affected by textile dyes and auxiliaries.

APPENDIX

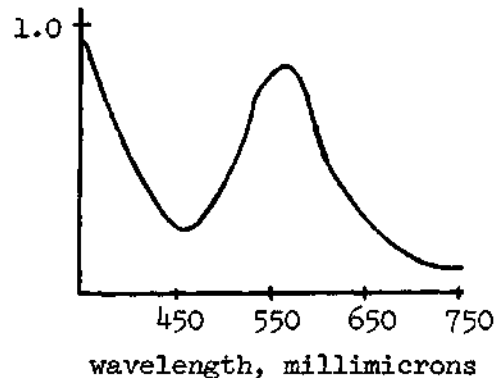
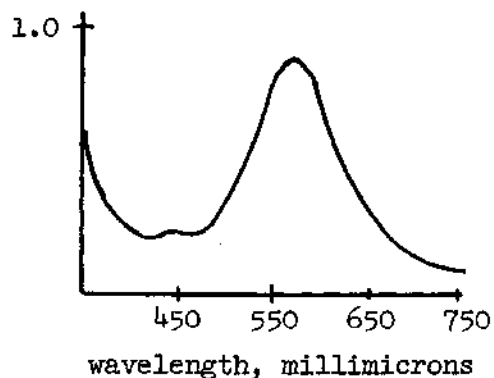
Dyes Dissolved in
Distilled Water

Dyes after 20 Days in
the Aerobic Digesters

(a) Reactive Blue 19



(b) Reactive Violet 5



(c) Reactive Black 5

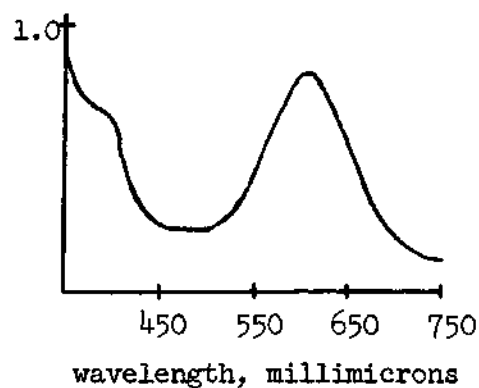
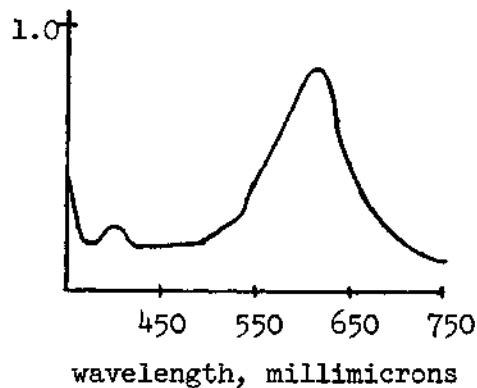


Figure 9. Visible Spectra of the Dyes Used for Aerobic Digestion

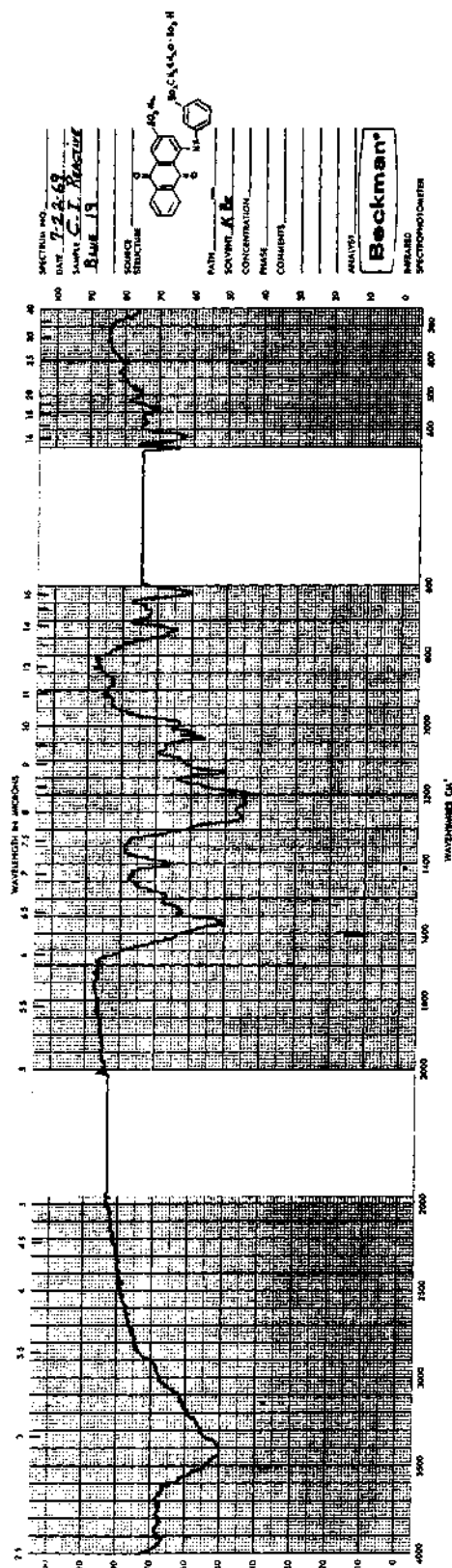


Figure 10. The Infrared Spectra of Reactive Blue 19

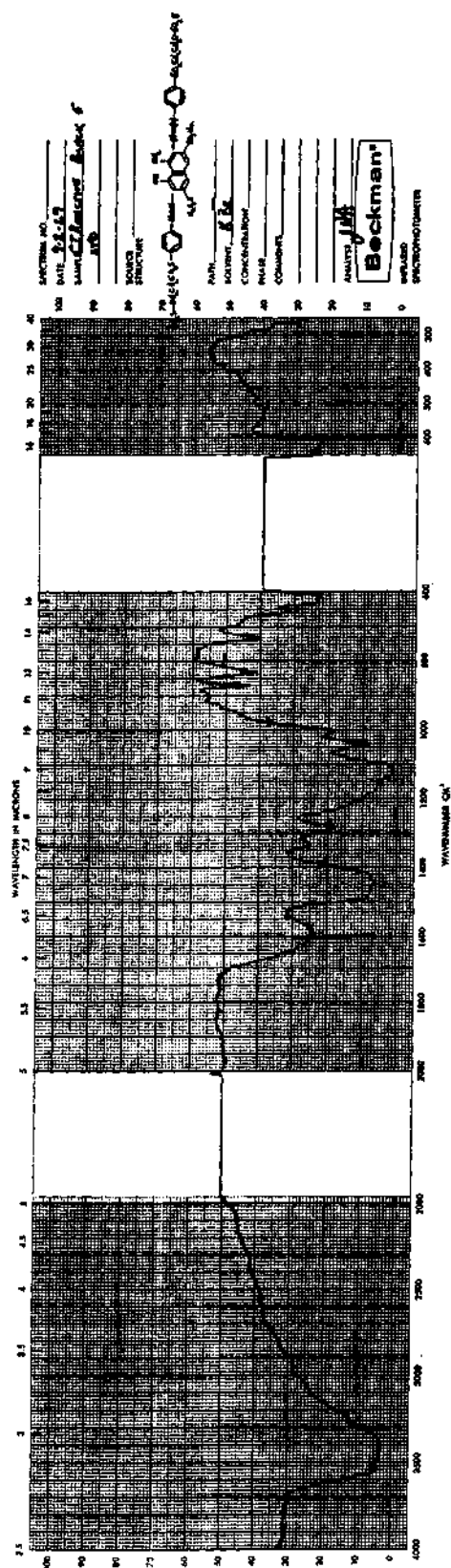


Figure 12. The Infrared Spectra of Reactive Black 5

Table 1. Daily Record of Aerobic Tank Containing 10 mg/l of Reactive Blue 19

Number of Days in Sludge	pH	Sludge Temperature (°C)	Sludge Color	Sludge Volume 1 hour Settling (ml)	Bacteria Count	Chemical Oxygen Demand (mg/l)
--	6.3	24.0	Brown	1200	104 x 10 ⁴	138
--	7.4	26.0	"	1200	---	189
*	8.1	24.0	"	1400	162 x 10 ⁵	388
1	8.2	24.0	"	900**		177
2	8.6	24.5	"	"	33 x 10 ⁵	160
3	8.6	24.5	"	"		224
4	8.5	24.5	"	"		200
5	8.5	24.5	"	"		193
6	8.2	24.0	"	"		174
7	8.2	24.0	"	"	49 x 10 ⁴	166
8	8.4	24.0	"	"		91
9	8.6	23.5	"	"		†
10	8.6	23.5	"	"		†
11	8.5	24.0	"	"		†
12	8.4	24.0	"	"	41 x 10 ⁴	89
13	8.6	24.0	"	"		106
14	8.8	23.5	"	"		147
15	8.8	23.5	"	"		174
16	8.8	23.5	"	"		135
17	8.7	23.5	"	"		127
18	8.7	23.5	"	"		116
19	8.7	23.5	"	"		98
20	8.7	23.5	"	"	144 x 10 ⁴	83

* 10 mg/l of dye added to tank over eight hour period.

** Sludge volume decreased to maintain between 20 and 30 percent of total tank volume.

† Unexplained increase in chemical oxygen demand--too high to measure.

Table 2. Daily Record of Aerobic Tank Containing 10 mg/l of Reactive Violet 5

Number of Days in Sludge	pH	Sludge Temperature (°C)	Sludge Color	Sludge Volume 1 hour Settling (ml)	Bacteria Count	Chemical Oxygen Demand (mg/l)
--	6.0	24.0	Brown	1100	137 x 10 ⁴	154
--	6.8	26.0	"	1100		213
*	8.2	24.0	"	1400	140 x 10 ⁵	428
1	8.4	24.0	"	900**		157
2	8.6	24.5	"	"	39 x 10 ⁵	160
3	8.6	24.5	"	"		180
4	8.6	24.5	"	"		194
5	8.6	24.5	"	"		200
6	8.6	24.0	"	"		202
7	8.5	24.0	"	"	127 x 10 ⁴	320
8	8.2	24.5	"	"		107
9	8.3	24.0	"	"		†
10	8.3	23.5	"	"		†
11	8.4	24.0	"	"		†
12	8.4	24.0	"	"	88 x 10 ⁴	190
13	8.6	24.0	"	"		105
14	8.5	23.5	"	"		163
15	8.6	23.5	"	800		178
16	8.6	24.0	"	"		139
17	8.5	24.5	"	"		138
18	8.5	24.0	"	"		138
19	8.4	24.0	"	"		130
20	8.3	23.5	"	"	128 x 10 ⁴	127

* 10 mg/l of dye added to tank over eight hour period.

** Sludge volume decreased to maintain between 20 and 30 percent of total tank volume.

† Unexplained increase in chemical oxygen demand--too high to measure.

Table 3. Daily Record of Aerobic Tank Containing 10 mg/l of Reactive Black 5

Number of Days in Sludge	pH	Sludge Temperature (°C)	Sludge Color	Sludge Volume 1 hour Settling (ml)	Bacteria Count	Chemical Oxygen Demand (mg/l)
--	8.3	24.0	Brown	1200	88×10^5	427
*	8.6	24.0	"	1200	115×10^4	78
1	8.6	23.5	"	1500	115×10^4	93
2	8.8	23.5	"	1600		111
3	8.8	24.0	"	1600		107
4	8.8	24.0	"	1800		150
5	8.8	24.0	"	1000**		167
6	8.7	23.5	"	"		183
7	8.7	23.5	"	"	33×10^5	238
8	8.6	23.5	"	"		176
9	8.5	23.5	"	"		162
10	8.4	23.5	"	"		128
11	8.4	24.0	"	"		124
12	8.4	24.0	"	"		120
13	8.4	23.5	"	"		97
14	8.5	23.5	"	"		62
15	8.1	23.5	"	"	33×10^5	146
16	8.0	23.5	"	"		169
17	8.0	22.5	"	"		154
18	7.8	22.5	"	"		148
19	7.7	23.0	"	"		140
20	7.4	23.5	"	"	240×10^4	125

* 10 mg/l of dye added to the tank over an eight hour period.

** Sludge volume decreased to maintain between 20 and 30 percent of total tank volume.

Table 4. Daily Records of Anaerobic Digesters

Number of Days in Digesters	Room Temp. (°C)	Control Tank			Reactive Blue 19 Tank				Reactive Violet 5 Tank			
		pH	Gas Volume (ml)	pH	Total Gas Volume (ml)	Δ Gas Volume (ml)*	Dye Added (mg/l)	pH	Total Gas Volume (ml)	Δ Gas Volume (ml)	Dye Added (mg/l)	pH
--	24.0	7.2	---	7.2	---	---	--	7.4	---	---	--	7.4
--	24.0	6.0	33	6.0	33	---	--	6.1	33	---	--	6.1
--	24.0	5.8	25	5.7	23	---	--	5.8	25	---	10	5.8
1	23.5	5.6	37	5.5	36	0	10	5.5	34	-3	10	5.5
2	23.0	5.5	37	5.4	30	-7	10	5.4	30	-7	10	5.4
3	25.0	5.4	65	5.4	54	-11	10	5.4	53	-11	10	5.4
4	24.0	5.2	31	5.2	28	-3	10	5.2	27	-4	10	5.2
5	24.5	5.2	37	5.1	43	5	10	5.1	35	-2	10	5.1
6	23.5	5.1	75	5.1	113	38	10	5.1	97	22	10	5.1
7	23.5	5.1	126	5.2	227	101	10	5.1	158	32	10	5.1
8	24.5	5.3	256	5.3	296	40	10	5.3	282	26	10	5.3
9	24.0	5.3	259	5.3	300	41	10	5.4	290	31	10	5.4
10	24.0	5.4	262	5.3	305	43	--	5.3	294	32	--	5.3

* Δ Gas volume = the total gas volume of dye tank minus the total gas volume of control tank.

Table 5. R_f Values of Metabolites Obtained from the Anaerobic Biodegradation of Reactive Blue 19

Color of Metabolite	Approx. % of Total	R_f Value	Remarks
Orange	> 1	1.0	Fluorescent under ultraviolet-water soluble
Orange	>> 1	2.0	" " " "
Pale Violet	> 1	3.0	" " " "
Yellow	>> 1	4.4	" " " "
Red Violet	98 +	5.3	Non fluorescent--water insoluble

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